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A Novel Electrochemical Sensor for Determination of Sildenafil Citrate (Viagra) in Pure Form and in Biological and Pharmaceutical Formulations

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A rapid and simple method was developed for sildenafil citrate (SILC) drug (the active component of Viagra) determination by electrochemical analysis using a screen-printed glassy carbon electrode (SPGCE). Initial investigations were undertaken using cyclic voltammetry (CV) to characterize the redox behavior at the SPCE. The respective current response has been evaluated with respect to the drug composition, pH of the supporting electrolyte, potential and scan rate. In addition, square wave voltammetry (SWV) was carried out to ascertain whether trace amount of samples could be detected. From these studies it was shown that the electrode exhibited linear response in the concentration range from 1.0×10^{-6} to 1.4×10^{-5} molL^{-1} with good reproducibility. The limit of detection was found to be 5.5×10^{-8} molL^{-1}. An interference study was also carried in presence of high concentration of ascorbic acid (AA) and uric acid (UA) to estimate the high selectivity of the electrode. The method was successfully applied for the determination of SILC in spiked human urine and pharmaceutical samples with good agreement between the added and recovery values.

Keywords: sildenafil citrate (Viagra), screen printed glassy carbon electrode (SPGCE), cyclic voltammetry, biological samples.

1. INTRODUCTION

A reliable and specific assay is of great importance for characterization of a drug's disposition, tolerance and safety. Viagra (SILC), widely used in oral therapy for erectile dysfunction, is the citrate salt of sildenafil, a selective inhibitor of cyclic guanosine monophosphate (cGMP) specific phosphodiesterase type 5 (PDE5) [1–4]. This drug can also be efficient as therapy for a range of cardiovascular diseases, such as pulmonary arterial hypertension (PAH) [5–10]. Sildenafil citrate is a

compound of the pyrazolo-pyrimidinylmethylpiperazine Class which is designated chemically as 1-[[3-(6,7 – dihydro – 1 – methyl – 7 – oxo – 3 – propyl – 1 – H – pyrazolo [4,3-d] pyrimidin -5-yl)-4-ethoxyphenyl]sulfonyl]-4-methyl-piperazine citrate (Fig. 1). The mode of action of sildenafil in the erection of the penis involves the release of nitric oxide (NO) in the corpus cavernosum during sexual stimulation. The produced NO activates the enzyme guanylate cyclase, which results in increased levels of cGMP, producing smooth muscle relaxation of the penile in the corpus cavernosum and therefore having the potential to improve penile erectile function by allowing inflow of blood [11, 12].

NO may also participate in disease processes such as hypertension, diabetes, impotence and stroke. Thus, NO can be toxic or beneficial depending on the amount and where in the body it is released [13]. SILC has a spillover effect and blocks PDE-6 enzyme, which cause an increase the concentration of cGMP and result in a 'blue vision'. Also, oral administration of SILC with other drugs like nitrates or nitroglycerine or isosorbide can induce headaches and low blood pressure [14]. Thus, the residual presence of SILC even in trace (nanomolar) concentration levels could result in adverse side effects. Therefore, a sensitive method for the selective determination of SILC in nanomolar level is required.

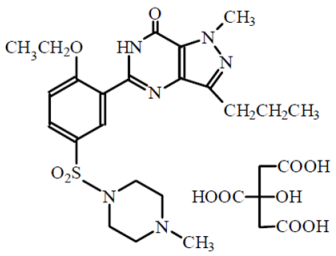


Figure 1. Structure of sildenafil citrate

Some methods have been reported for determination of sildenafil citrate including spectrophotometry based on formation of ion-associate complexes with dyes [15-17], flow injection analysis with UV detection [18], extractive spectroscopy [19], HPLC [20], and resonance Rayleigh-scattering [21]. Most of these methods are expensive, suffer from lack of selectivity and require careful control of conditions and considerable time for routine control analysis [22, 23]. Other methods have been reported for electrochemical determination of SILC, which can generally be based on either reduction [24, 25], or oxidation [26, 27]. Most of the electrochemical determination has been reported for SILC at a hanging mercury drop electrode (HMDE) [28]. However, the use of HMDE is undesirable because of the high toxicity of mercury and the inherent problems associated with HMDE, such as oxygen removal, cleaning the surface of mercury drop. A new methodology based on "mercury free" electrode is desirable.

Moreover, new electrochemical instrumentation is compact and portable, allowing on-site analysis and obtaining results in only minutes without preliminary sample treatment. In the development of electrochemical devices, screen printed electrodes which can be ideally used for voltammetric measurements using portable electrochemical instrumentation [29–34]. The main advantages of this kind of electrode system are associated with their modest cost, potential portability,

simplicity of operation, reliability, and the compact detector arrangement containing the working electrode, auxiliary and reference electrodes. Importantly, the low cost of the electrodes means that it is possible to use a new electrode for each measurement thereby avoiding possible poisoning of the electrode surface by the product of the electrochemical measurement. The effective performance of screen-printed electrodes has led to their widespread acceptance in environmental, biomedical, occupational hygiene monitoring and all major fields of analytical chemistry [35–38].

The aim of this study was to optimize and develop a sensitive, fast and accurate adsorptive voltammetric method for the determination of SILC in biological and pharmaceutical formulations.

2. EXPERIMENTAL

2.1. Materials and reagents

All chemicals were used as received without further purification. Sildenafil citrate was supplied from Pfizer, uric acid and ascorbic acid were supplied by sigma Aldrich Company. Britton–Robinson (B-R) buffer (pH 1.6–9.3) was prepared from 0.12 M CH_3COOH, 0.12 M H_3BO_3 and 0.12 M H_3PO_4, and adjusted with 0.5 M NaOH. Aqueous solutions were prepared using double distilled water.

2.2. Apparatus and measurements

Voltammetric measurements were carried out with a mini Autolab PGSTAT 910 potentiostat connected to a personal computer. The measurements were performed in a specially fabricated wall-jet cell containing the electrode strip. Screen-printed glassy carbon electrode (SPGCE) strips were purchased from Metrohm. The design of the SPGCE used in all these electrochemical experiments is shown in Fig. 2.

The electrode is based on an alumina ceramic base(s) 35 mm long, 10 mm wide and 0.45 mm thick. On to this surface the working (W), reference (R) and the auxiliary (A) electrode were applied.

SWV and CV were used for the determination of SILC using SPGCE electrode. Where the potential was scanned from potential −1.6 to 1.0 V, with scan rate of 100 mV s^{-1}.

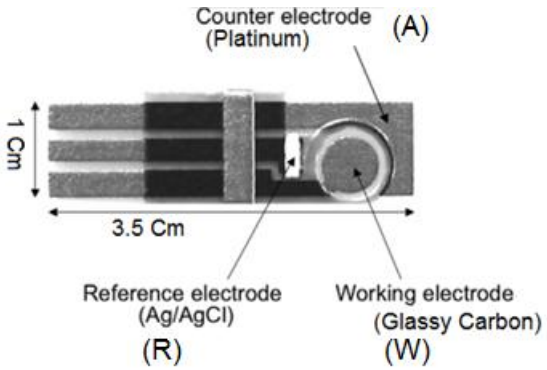


Figure 2. Image of the screen-printed glassy carbon strip (SPGCS) consisting of a Glassy carbon working electrode (W), a platinum counter electrode (A), and Ag/AgCl reference electrode (R).

2.2. Analysis of urine and SILC tablets

The utilization of the proposed method in real sample analysis was also investigated by direct analysis of SILC in human urine samples as well as in its pharmaceutical formulation. For this purpose, the urine samples were diluted 10 times in B-R buffer (pH 4.5) to minimize any matrix effect. In 10 mL measuring flasks, three different amounts of 0.5 $mmolL^{-1}$ SILC solution were added to 2.0 mL of urine sample, diluted with B-R buffer, poured into the electrolytic cell, and the corresponding SWVs were recorded. Regarding the pharmaceutical formulation of SILC, 5 x 100 mg tablets of Viagra were weighed and then the average mass per tablet was determined. The tablets were carefully grounded to a fine powder, and then a quantity of homogeneous powder equivalent to 50 mg of SILC was dissolved in 100 mL of water by sonication for 10 min, followed by mechanical shaking for about 10 min. The desired concentration of SILC was obtained by accurate dilution with B-R buffer. The sample solution so prepared was added to the supporting electrolyte in the voltammetric cell and SWV of the solution was recorded and the anodic peak current was evaluated. Furthermore, the electrode was applied for the recovery assessment of SILC in the tablets by the standard addition method. In this respect, different standard concentrations of SILC were added to the tablets solution.

3. RESULTS AND DISCUSSION

3.1. Electrochemical behavior of SILC at SPGCE

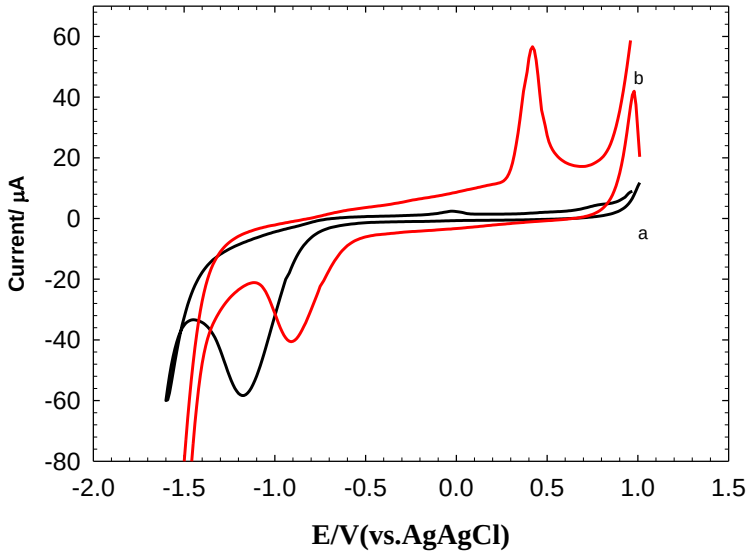


Figure 3. CVs of SPGCE in B-R (pH 4.5) (a) in absence, (b) in presence of 0.5 $mmolL^{-1}$ of SILC.

Fig. 3 (curve a) shows cyclic voltammogram of 0.5 $mmolL^{-1}$ SILC in 0.12 M B-R buffer pH 4.5 obtained using SPGCE in the potential range of 1.0 to –1.6 V. The base voltammogram is also depicted for comparison Fig. 3 (curve b). A high response of current is obtained at 0.43 V, indicated

that SILC undergoes an oxidation on SPGCE and the redox reaction is totally irreversible. Sildenafil containing basic functional groups with a pK_{a2} value of 8.7 has a weak acidic moiety. In the substituted and fused rings of pyrimidine and pyrazol, protonation is highly difficult due to resonance and steric effects. Therefore, the only site in sildenafil vulnerable for protonation is the nitrogen bonded to electron-donating methyl group in the piperazine ring [39-41] giving formyl piperazine which is responsible for the appearance of this oxidation peak.

3.2. Effect of potential on the oxidation peak

Fig. 4 shows cyclic voltammograms of 5 $mmolL^{-1}$ SILC in 0.12 M B-R buffer pH 4.5 obtained using SPGCE at different potential ranges from 0.0 to −1.6V. No oxidation peak is observed by scanning from 0.0 V to −1.5 V, at lower potential than that the oxidation peak began to be noticed with higher current intensity, indicating the oxidation of SILC by going more negative potential.

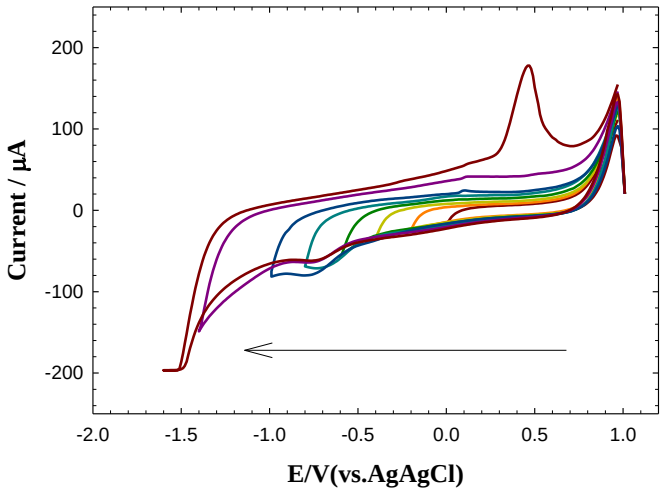


Figure 4. CVs of SPGCE in B-R (pH 4.5) at different potential values in the presence of 5 $mmolL^{-1}$ of SILC.

3.3. Effect of pH

The influence of pH on the voltammetric response of SILC was studied. In order to establish a suitable pH, a range of values was examined between pH 1.6 and 9.3. The voltammograms obtained for the different pH values are presented in Fig. 5A.

There are three main groupings present in the structure of SILC which might be considered as under-going electro-oxidation: the citrate anion, the *p*-alkoxybenzene-sulfonamide grouping and the piperazine ring. It was shown that SILC has two ionization constants. The first constant (pK_{a1}) is a characteristic of an acidic group and the second constant (pK_{a2}) was attributed to the basic group. The two pK_a values for SILC are 5.5 and 8.7 for the ionization sites, respectively. Dependence of the

oxidation peak currents on pH tested in 0.5 mmolL^{-1} solutions of SILC is shown Fig.5B. In the measurement of solutions adjusted at pH < 5.5, oxidation current signals for SILC were observed in which the peak potentials remain almost fixed. Thus, the oxidation potential remains pH-independent at pH < pK_{a1}. A very good current response with a sharp peak was obtained at pH 4.5 Fig. 5B. This implies on the involvement of protonation/deprotonation in the redox process of SILC. The monoprotonated form of the piperazine ring, rapidly generated from the dication, is oxidized at pH < pK_{a1}. For this reason a value of pH 4.5 allowed the most favorable condition to carry out the study and thus the methods were developed at this pH value.

The sharp form of this peak already indicates that adsorption is involved. This has been confirmed by repeated cycles in SILC (Fig. 6). The anodic current peak increases as the number of repeated cycles increases.

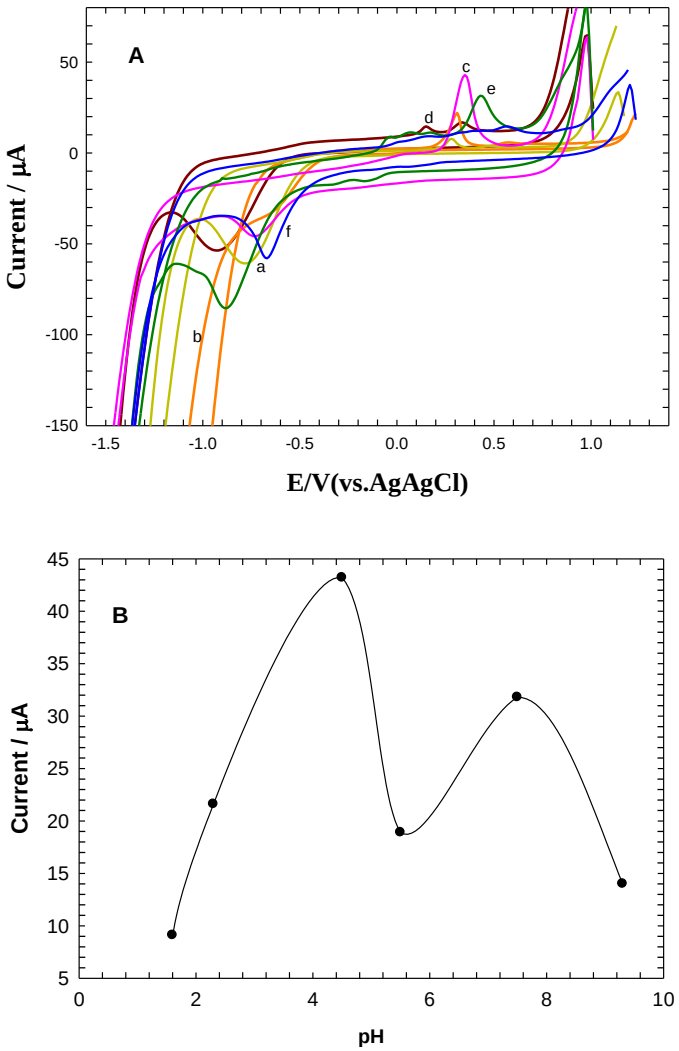


Figure 5. CVs of SPGCE at different pH values (a) pH 1.6, (b) pH 2.3, (c) pH 4.5, (d) pH 5.3, (e) pH 7.5, (f) pH 9.6. (A). Dependence of the peak current on pH in 0.5 mmolL^{-1} solutions of sildenafil (B). Scan rate: 100 mVs^{-1}.

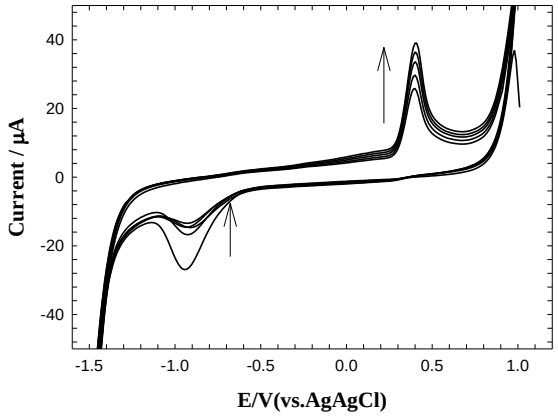


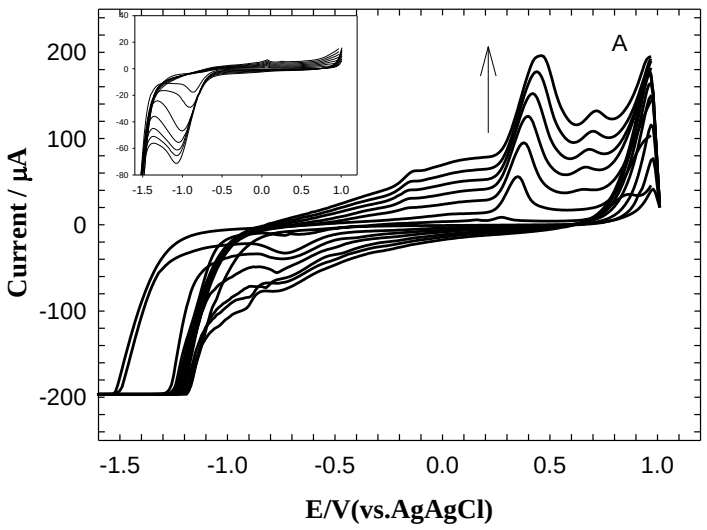
Figure 6. CVs for repeated cycles of SPGCE in the presence of 0.5 mmolL^{-1} of SILC

Around pH 7, a broad anodic peak was observed with a small shoulder, and a rising of two small reduction peaks, which can be regarded to the adsorption of both the protonated form of the oxidation product of SILC and its conjugate base. The conjugate base of the oxidation product is more strongly adsorbed [40].

3.4 Effect of scan rate

The oxidation peak currents (I_p) of SILC at SPGCE in presence of 0.5 mmol L^{-1} SILC solutions (pH 4.5) varied with change of square root of scan rate (v) in the range of 20–120 mVs^{-1} as shown by the cyclic voltammograms in Fig.7A. The higher the scan rate, the greater is the peak current.

The anodic peaks current and peak potential were proportional to the square root of scan rate in the range of 20–120 mVs^{-1} (Figure 7B and C).



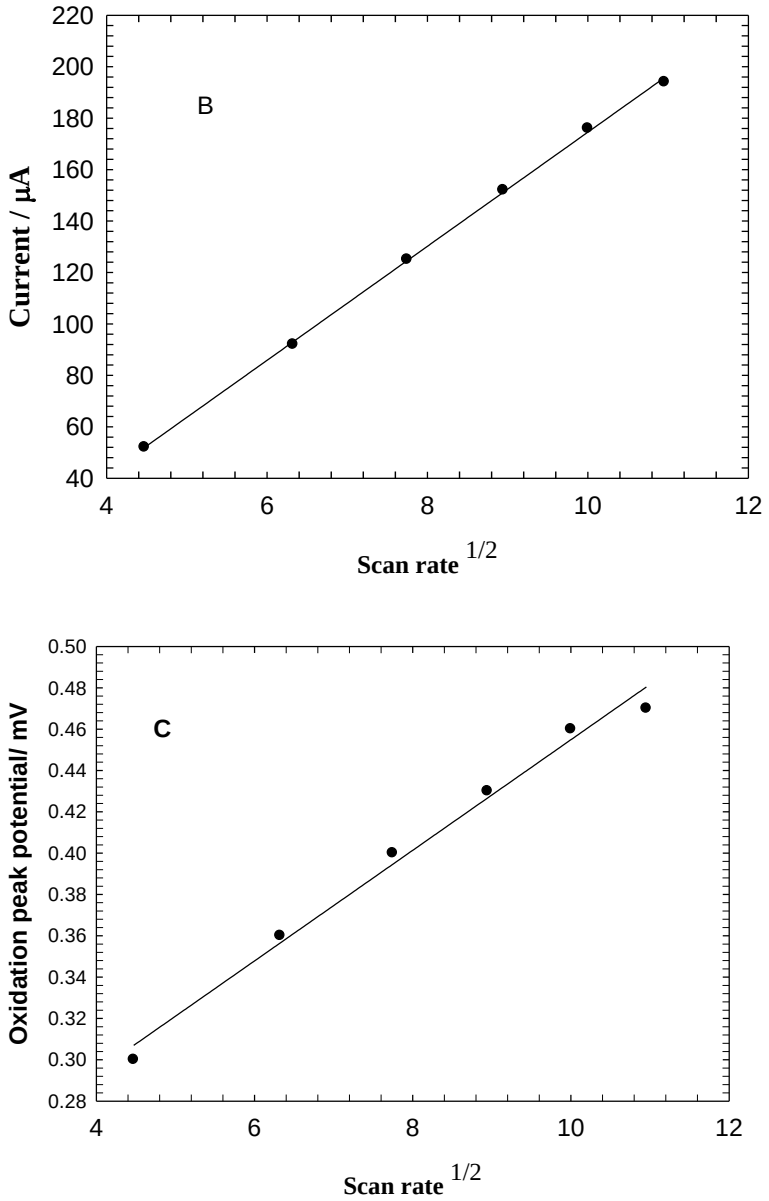


Figure 7. Cyclic voltammograms of 0.5 mmolL^{-1} SILC in 0.1M B–R buffer solution pH 4.5 at different scan rates (20, 40, 60, 80, 100 and 120 mVs^{-1}), inset in absence of SILC (A), Dependence of the oxidation peak Current (B), potential (C) on square root of scan rate of 0.5 mmolL^{-1} SILC solution.

A linear relationship was observed between the peak intensity I (in µA corresponding to the oxidation process) and the square root of the scan rate, V, demonstrating that the phenomenon is adsorption-controlled with the regression equation:

$$I_p\ (\mu A) = 3.2 + 0.22\ v^{1/2}\ (mV\ s^{-1}) \qquad (r = 0.998)$$

At relatively slow voltage scans, the adsorbed layer grows much further towards the solution side and further from the electrode surface. Therefore, as the scan rate increases the flux to the

electrode surface increases considerably. At relatively higher scan rates the adsorbed layer grows less further from the vicinity of the electrode.

3.5. Calibration graph and limit of detection

Considering the larger response obtained for SILC by SWV (Fig.8). It was expected that this technique to be successfully applied for the determination of Viagra. The corresponding calibration graph for oxidation peak was linear from 1.0×10^{-6} to 1.4×10^{-5} molL^{-1} and obeyed the equation I = 0.15c + 0.035, where I and c are the peak current (μA) and SILC concentration (μmolL^{-1}), respectively. The correlation coefficient (r) was 0.998. The reproducibility of the square -wave signal expressed in terms of the relative standard deviation, at a concentration level of 5×10^{-6} molL^{-1} was 4.8% (n=3). The detection limit for oxidation peak based on the 3σ criterion was estimated as 5.5×10^{-8} molL^{-1}.

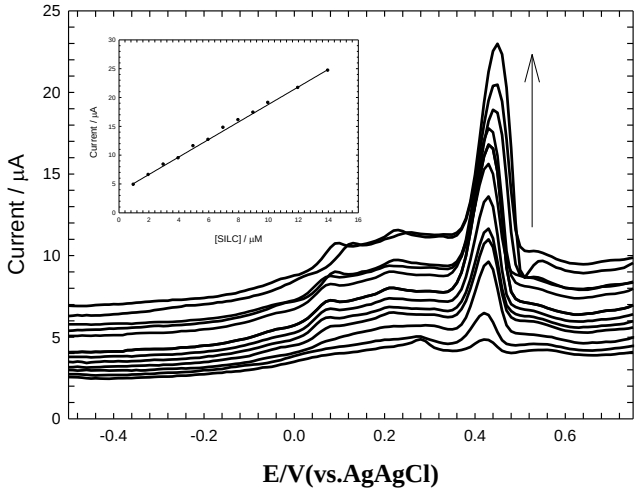


Figure 8. SWVs for successive additions of SILC in the concentration range from 1×10^{-6} to 1.4×10^{-5} molL^{-1}. The inset shows the corresponding calibration plot.

3.6. Electrochemical interference studies for SILC at SPGCE

The selectivity of the electrode towards SILC was evaluated by the successive addition of SILC from 0.5 mmolL^{-1} stock solution to possible interferents (uric acid and ascorbic acid). SWV voltammograms were recorded at SPGCE for a mixture of 50 mmolL^{-1} ascorbic acid, 5 mmolL^{-1} uric acid. It has been noticed that at B-R solution of pH 4.5, ascorbic acid has no oxidation reduction peak at SPGCE, while uric acid has an oxidation peak at 0.19 V. By successive addition of SILC solution an oxidation peak was noticed at 0.47 V, which increases by increasing the concentration of SILC. Altough, the concentration of ascorbic acid and uric acid are much higher than that of SILC by 1000 and 100 times, respectively, the SPGCE still can sense the lower concentrations of SILC.

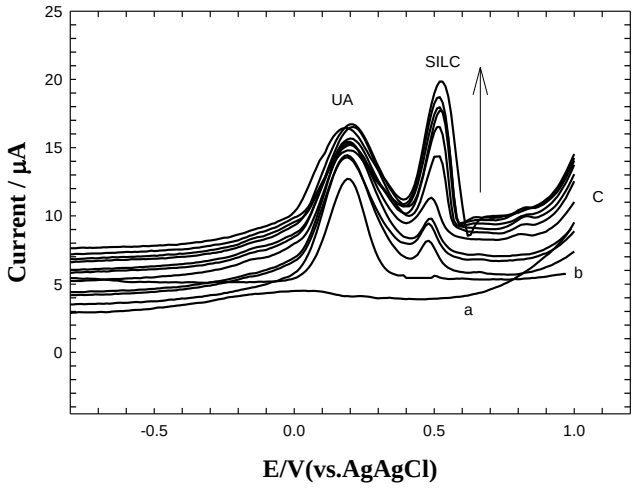


Figure 9. SWVs for SPGCE in B-R (a), SPGCE in a mixture of 50 mmolL^{-1} ascorbic acid, 5 mmolL^{-1} uric acid (b) and in successive addition of 0.5 mmolL^{-1}SILC in the same mixture (c) with scan rate 100 mVs^{-1}, pH 4.5.

3.7. Analytical Applications

3.7.1. Determination of SILC in human urine

The proposed method was used to detect SILC in urine samples, which obtained from healthy volunteer. No signal was observed for SILC in urine samples; therefore, the urine samples were spiked by different concentrations of SILC standard solution, and then used for further determination. The voltammograms were recorded using the SWVs, and the corresponding values were recorded. The results obtained are given in Table 1. As can be seen for the determination of SILC, good recoveries were obtained ranging from 98.5-101.4%.

Table 1. Recoveries in spiked human urine samples

Urine sample	Spike (µmol L^{-1})	Found (µmol L^{-1})	Recovery (%)	R.S.D. (%)[a]
1	4.00	3.94	98.5	2.4
2	8.00	8.11	101.4	2.3
3	12.00	12.02	100.2	2.9

[a] average of 3 times repetition

3.7.2. Determination of SILC in pharmaceutical tablets

SPGCE are successfully applied for the determination of SILC in pharmaceutical preparations by standard addition method. These results indicated that there is no interference from the tablet

coating materials, or fillers. The results depicted in Table 2 are in good agreement with the claimed values with average recovery of 99.5%.

Table 2. Tablet results and recoveries obtained for four determinations of SILC in spiked Viagra tablets

sample	content ($\mu mol\ L^{-1}$)	SILC added ($\mu mol\ L^{-1}$)	SILC found ($\mu mol\ L^{-1}$)	Recovery (%)
1	5.00	2.00	7.15	102.14
2	5.00	4.00	8.94	99.33
3	5.00	6.00	10.97	99.73
4	5.00	8.00	12.56	96.62

4. CONCLUSION

This work shows that the SILC can be determined using voltammetric techniques on the basis of piperazine ring oxidation process over SPGCE. This behavior provides a useful tool for its detection and quantification at low levels of concentration. A faster analysis can be performed by direct measurement from the calibration graph established for SWV. This method is sensitive enough, and there is no interference in the analysis from the other components (excipients) also present in the pills.

The proposed methods are simple, fast and low cost suitable for SILC analysis in human urine and pharmaceutical formulations. The recovery results prove that the proposed procedures are sufficiently accurate and precise and can be applied to pharmaceuticals.

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